Biosorption of Nickel by Yeasts in an Osmotically Unsuitable Environment

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The tolerance, sorption of nickel(II) ions, and changes in the production and composition of exopolymers of eight yeast strains grown under nickel presence with/without NaCl were studied. Strains of *Pichia anomala* and *Candida maltosa* known as the most resistant yeasts against nickel tolerated up to 3 mM Ni^{2+}. NaCl addition decreased both the resistance of the yeast strains toward nickel ions and the sorption of metal ions into cells. All yeasts absorbed nickel predominantly into exopolymers (glycoproteins) and on the surface of cells. However, while the amount of polysaccharide moieties of exoglycoproteins of most of the resistant yeasts was induced by stress conditions, the ratio polysaccharide/protein in the exopolymers remained unchanged in the sensitive species *Cystofilobasidium*. The exopolymer composition might play a key role in yeast adaptation to stress conditions caused by heavy metal ions.

Key words: Salt Stress, Nickel Stress, Exopolymers, Yeasts

Introduction

Nickel as a heavy metal is the fifth most abundant element on earth. It has many applications and usages, mainly in alloy preparations and as an ingredient of metal products. These and other industrial activities raise its level in the environment, leading to continuous introduction of this element into the food chain. Thus effective removal of nickel from the environment is an increasing human health concern (Cakmak *et al.*, 2000).

Recently, bioremediation by microorganisms as biosorbents has been applied successfully for the elimination of soluble pollutants (Davis *et al.*, 2003; Wong *et al.*, 2000; Ansari and Malik, 2007; Zafar *et al.,* 2007). Yeast cells have been considered not only as an useful tool for heavy metal removing from the environment but also as an excellent model for the study of the mechanisms underlying the tolerance to heavy metal stress (Serrano *et al*., 1997). Yeast cells can accumulate metal ions in two general manners: (1) non-metabolic adsorption/binding of metal ions onto the cell surface and (2) metabolic accumulation in the cytosol (Murray and Kidby, 1975; Norris and Kelly, 1977; Gadd, 1983, 1990). Extracellular yeast polymers, such as polysaccharides and glycoproteins, also play an important role in non-metabolic metal adsorption (Gadd, 1983; Breierová *et al.*, 2004).

Nickel is an essential nutrient for some microorganisms, although toxic at higher concentrations (Joho *et al.*, 1995; Farcasanu *et al.*, 2005). Therefore, increased biosorption of Ni at higher concentrations in the environment could be activated by changing the microbial growth conditions. One of the methods is based on application of osmotic stress; then yeast cells produce and accumulate osmoprotectants, so-called compatible solutes (Hohmann, 2002; Breierová *et al*., 2004). Because the presence of nickel as pollutant in the environment is often accompanied by elevated salt concentrations, the aim of this work was focused on determining how osmotic stress affects the nickel sorption by selected yeast species.

Materials and Methods

Microorganisms

The following yeast species were used: *Aureobasidium pullulans* CCY 27-1-111, CCY 27-1-115; *Debaryomyces castellii* CCY 41-9-11; *Candida maltosa* CCY 29-88-15; *Pichia anomala* CCY 38-1-22; *Cryptococcus laurentii* CCY 17-3-16; *Cystofilobasidium capitatum* CCY 10-1-3, CCY 10-1-11.

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The strains have been stored at the Culture Collection of Yeasts (CCY, Institute of Chemistry, SAS, Bratislava, Slovakia) at 4 °C on malt extract agar.

Cultivation conditions

All strains grew at optimal temperature in 500 ml flasks with 250 ml cultivation medium on an orbital shaker (80 cycles min^{-1}). The basal optimal medium used for cultivation contained: 4 g l^{-1} yeast extract; 10 g l^{-1} $(NH_4)_2SO_4$; 20 g l^{-1} glucose; 1 g l^{-1} KH_2PO_4; 0.2 g l^{-1} $K_2HPO_4 \cdot 3H_2O$; 0.1 g l^{-1} NaCl; 0.1 g l^{-1} $CaCl_2$; 0.5 g l^{-1} $MgSO_4 \cdot 7H_2O$; and 1 ml l^{-1} microelement solution: 1.25 mg l^{-1} H_3BO_4; 0.1 mg l^{-1} $CuSO_4 \cdot 5H_2O$; 0.25 mg l^{-1} KI; 1 mg l^{-1} $MnSO_4 \cdot 5H_2O$; 0.5 mg l^{-1} $FeCl_3 \cdot 6H_2O$; 0.5 g l^{-1} $(NH_4)_2Mo_7O_{24} \cdot 4H_2O$ and 1 g l^{-1} $ZnSO_4 \cdot 7H_2O$. The rest chemicals were purchased from Lachema Brno (Czech Republic). The cultures were incubated until the end of the exponential growth phase. The osmotic and heavy metal stress were induced by addition of different concentrations of NaCl and nickel ions to the basal cultivation medium, respectively. For each strain non-lethal and simultaneously maximum tolerated concentrations (sublethal) for both stress substances and their combination were verified by the measurement of the optical density (OD 660 nm) during their growth; the maximum metal ion or NaCl concentrations at which they yeast growth was detected are shown in Tables I and II.

Sample preparation

Yeast cells were harvested in the late exponential growth phase by centrifugation (4 °C, for 10 min at 3000 × *g*). Supernatant containing exopolymers was precipitated with two volumes of 96 % ethanol and centrifuged. The precipitate was dissolved in distilled water, exhaustively dialyzed against distilled water, and the exopolymers were freeze-dried (Breierová *et al*., 1996).

Isolated cells were resuspended in distilled water and subjected to ultrasound treatment (Person-Ultragen UZD 300, Nitra, Slovakia) at 20 kHz, 21 °C and 110 W for 3 × 2 min (Stratilová *et al.*, 1998). The cells were separated by centrifugation and the fibrillar part of the cell walls was obtained from the supernatant by precipitation with 96 % ethanol (1:2 v/v). The sediment was frozen by liquid nitrogen. After defrosting, the cytosol material was isolated from the supernatant by ethanol precipitation and the sediment was used as cell material. All samples were freeze-dried and analyzed. The resulting data represent the mean value from three independent experiments.

Analytical methods

The carbohydrate content was measured by the phenolsulfuric acid method at 490 nm (Dubois *et al.,* 1956) with D-glucose as standard. Proteins were estimated by Lowry's method using bovine serum albumin as standard (Lowry *et al.*, 1951). Determination of Ni^{2+} ions was performed by the method of inductively coupled plasma-optical emission spectrometry (ICP-OES) in axial configuration. The microwave module disintegrated the lyophilized samples, and the Ni^{2+} ions were determined by a flame atomic absorption spectrometer with background correction by a deuterium lamp. An acetylene/air flame was used in the ratio of 1:1.2 with a fuel flow of 1.2 l min^{-1} for Ni^{2+} and measured at 232.0 nm.

Results and Discussion

Eight yeast strains belonging to three ascomycetous (*Debaryomyces castellii, Candida maltosa*, *Pichia anomala*) and three basidiomycetous species (*Aureobasidium pullulans*, *Cryptococcus laurentii, Cystofilobasidium capitatum*) were tested for their ability to accumulate nickel in hypertonic medium. It is known that nickel traces are necessary for functioning of several enzymatic processes in microbial cells. However, high levels are extremely toxic to living organisms resulting in inhibition of metabolic activities and cell growth (Dönmez and Aksu, 2001; Vadkertiová and Sláviková, 2006). Our study clearly showed resistance variations of the investigated strains against Ni. The tolerance increased from 0.5 mM Ni^{2+} (both *Cys. capitatum* strains) through 1 mM Ni^{2+} (*D. castellii, Cr. laurentii*), 2 mM Ni^{2+} (both *A. pullulans* strains) up to 3 mM Ni^{2+} (*C. maltosa*, *P. anomala*). By nickel stressed cells were significantly larger than cells in optimal conditions. Similar metal-induced morphological changes were also observed by Tuszyňska *et al.* (2006). The sensitivity or resistance of the yeasts to Ni^{2+} ions (and other metal ions) is associated with the production of metal-binding exopolymers and with the change in the fibrillar part of the cell wall (Blackwell *et al.*, 1995; Strouhal *et al.*, 2003; Breierová *et al.*, 2004). It should be noted that ascomycetous strains of yeasts were

Table I. Accumulation of Ni^{2+} ions in the compartments of yeast cells and exopolymers without addition of NaCl.

Strain CCY No.	Fibrillar part of wall	Cytosol	Cell	Exopolymers	$NiCl_2$ in medium [mM]
	% Ni^{2+} (w/w)[a]				
29-88-15	33.7 ± 0.4	41.3 ± 0.2	4.1 ± 0.1	20.9 ± 0.2	3
38-1-22	28.2 ± 0.2	48.0 ± 0.5	6.5 ± 0.2	17.3 ± 0.2	3
27-1-111	20.0 ± 0.2	18.0 ± 0.4	2.0 ± 0.1	60.0 ± 0.4	2
27-1-115	24.1 ± 0.3	7.7 ± 0.1	1.9 ± 0.1	66.3 ± 0.5	2
41-9-11	21.3 ± 0.2	33.8 ± 0.3	6.0 ± 0.2	38.9 ± 0.3	1
17-3-16	13.8 ± 0.1	13.8 ± 0.2	5.7 ± 0.2	67.7 ± 0.6	1
10-1-3	10.4 ± 0.1	2.1 ± 0.1	7.1 ± 0.3	80.4 ± 0.5	0.5
10-1-11	12.1 ± 0.2	2.3 ± 0.1	5.7 ± 0.2	79.9 ± 0.4	0.5

[a] Data represent mean percentage values ± SD of three independent experiments. Percentage values were calculated from total means sorbed by yeast cells and exopolymers.

Table II. Accumulation of Ni^{2+} ions in the compartments of yeast cells and exopolymers with addition of NaCl.

Strain CCY No.	Fibrillar part of wall	Cytosol	Cell	Exopolymers	$NiCl_2$/NaCl in medium [mM/%]
	% Ni^{2+} (w/w)[a]				
29-88-15	22.9 ± 0.3	42.8 ± 0.4	5.6 ± 0.1	28.7 ± 0.3	2/8
38-1-22	27.0 ± 0.2	15.6 ± 0.3	12.1 ± 0.2	45.4 ± 0.4	2/10
27-1-111	19.4 ± 0.1	14.4 ± 0.2	2.2 ± 0.1	63.9 ± 0.5	1/8
27-1-115	20.3 ± 0.3	19.9 ± 0.3	12.6 ± 0.3	47.2 ± 0.4	1/8
41-9-11	13.1 ± 0.2	24.2 ± 0.4	16.4 ± 0.3	46.3 ± 0.3	0.6/8
17-3-16	8.9 ± 0.2	5.6 ± 0.1	8.7 ± 0.2	76.8 ± 0.2	0.6/8
10-1-3	6.4 ± 0.1	5.7 ± 0.2	4.9 ± 0.2	83.0 ± 0.3	0.4/3
10-1-11	10.9 ± 0.2	4.8 ± 0.1	4.2 ± 0.1	80.1 ± 0.4	0.3/3

[a] Data represent mean percentage values ± SD of three independent experiments. Percentage values were calculated from total means sorbed by yeast cells and exopolymers.

more resistant to Ni^{2+} basidiomycetous species. This fact might be related to the different structure of both cell walls and exopolymers produced by yeasts in the cultivation medium. In general, the cell walls and exopolymers of yeasts are composed largely of polysaccharide-protein complexes which are also involved in heavy metal sorption. Distribution of Ni^{2+} ions in the individual cell compartments and exopolymers after their growth without and with NaCl is presented in Tables I and II. All studied strains absorbed nickel predominantly into exopolymers and on the surface of the cells. The strains of *P. anomala* and *C. maltosa* as the most resistant yeasts against nickel produced phosphomannans as component of exopolymers (Breierová *et al.*, 2002). Oppositely, both strains of *Cys. capitatum* showed high sensitivity towards nickel and osmotic stress. Also, these yeasts displayed high sensitivity to other metals (Vadkertiová and Sláviková, 2006). Our study illustrated, that NaCl addition to the cultivation medium reduced the resistance of the yeast strains toward nickel and simultaneously dramatically decreased the uptake and sorption of nickel ions into yeast cells (Fig. 1). A similar behaviour was also reported for algae (Davis *et al.*, 2003).

The surface of a cell plays an important role concerning the relationship between the cell and its environment because the surface is in direct contact with the ambient environment of the cell. Moreover, both essential (*i.e.*, nontoxic) and nonessential (*i.e.*, toxic) metal ions are transported across the surface into the cell (Collins and Stotzky, 1992). Stress conditions evoked a higher production of exopolymers as follows: control < osmotic stress < nickel stress < osmotic and

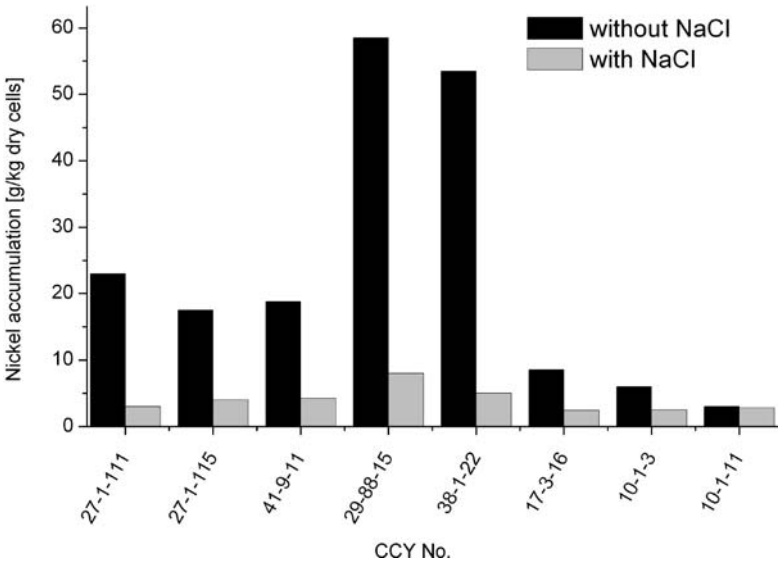

Fig. 1. Amount of nickel(II) ions accumulated in yeast cells in a suitable osmotic medium (without NaCl) and hypertonic medium (with NaCl).

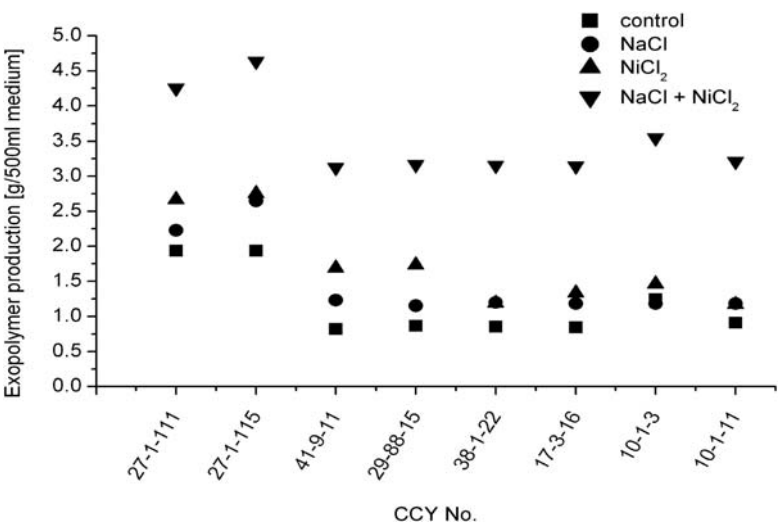

Fig. 2. Production of exopolymers by the studied strains under four different conditions of cultivation – control conditions, with NaCl, with $NiCl_2$, and with NaCl and $NiCl_2$.

nickel stress. It is evident that a combination of stresses caused elevated amounts of exopolymers produced by yeast strains (Fig. 2). These exopolymers and the cell surface (fibrillar part of wall) serve as a crucial protective barrier against penetration of heavy metal ions into the cells and their subsequent damage (Gibbs and Seviour, 1998; Breierová *et al.*, 2004). The properties of this barrier are dependent on the composition and structure of the polymeric protective substances represented by the polymeric complex of carbohydrate and protein (Breierová *et al.,* 1996). It is evident

Table III. Content of protein (prot) moiety and saccharide (sach) moiety in produced exopolymers.

Strain CCY No.	Optimal		NaCl		$NiCl_2$		$NaCl + NiCl_2$	
	sach	prot	sach	prot	sach	prot	sach	prot
	% (w/w)		% (w/w)		% (w/w)		% (w/w)	
27-1-111	92.6	3.4	82.8	7.2	81.3	9.3	70.7	1.4
27-1-115	92.6	3.4	83.9	5.1	88.2	8.4	71.8	4.7
41-9-11	78.4	21.6	71.5	10.5	76.8	10.1	73.1	5.7
29-88-15	73.6	26.4	66.1	10.9	65.5	9.3	72.2	5.9
38-1-22	79.3	14.7	72.9	10.10	73.7	20.1	70.8	4.1
17-3-16	77.5	20.5	80.1	8.9	78.1	9.3	67.8	3.9
10-1-3	71.1	15.9	78.8	15.5	79.6	11.4	72.5	4.6
10-1-11	81.2	11.8	73.5	4.4	71.3	7.9	73.7	4.7

that the production of exopolymers is a response of the yeast cells to the unbalance of the environment. A comparison of saccharide and protein moieties of individual exopolymers produced by the studied strains showed that exopolymers formed by the most resistant strains *P. anomala* and *C. maltosa* are characterized by the highest metal binding capacity and elevated levels of polysaccharides (Table III). On the other hand the ratio of polysaccharides and proteins in the exopolymers of the sensitive strains of *Cys. capitatum* was not influenced by stress conditions. These results indicate that the exopolymers composition might play one of the key roles in adaptation of the studied yeasts to stress conditions caused by nickel and the presence of NaCl.

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